

STUDY OF ISOANTIGENS IN CERVICAL NEOPLASIA BY MCAR AND IMMUNOFLUORESCENCE TECHNIQUE

THESIS
FOR M. D. (PATHOLOGY),
BUNDELKHAND UNIVERSITY, JHANSI.



CERTIFICATE

This is to certify that the work of
DR. VANDANA GUPTA on "THE STUDY OF ISOMER-
TIGENS IN CERVICAL NEOPLASIA BY H.C.A.R.
AND IMMUNOFLOUORESCENCE TECHNIQUES" which
is being presented by her for M.D.
(PATHOLOGY) examination, has been conducted
under my personal guidance.

She has put in the necessary stay in
the department according to the University
regulations.

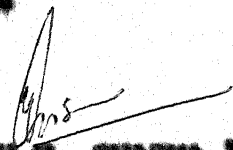
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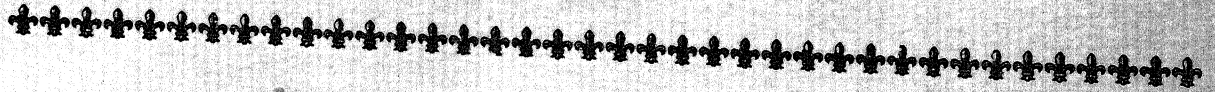
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CERTIFICATE

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DR. VANISHA GUPTA on "THE STUDY OF ISOEN-
ZYMES IN CERVICAL NEOPLASIA BY H.C.A.R.
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is being presented by her for M.D.
(PATHOLOGY) examination has been conducted
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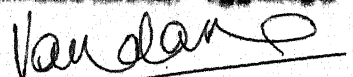
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C O N T E N T S

<u>CHAPTER</u>	<u>PAGES</u>
INTRODUCTION	1 --- 4
REVIEW OF LITERATURE	5 --- 21
MATERIAL AND METHOD	22 --- 29
OBSERVATIONS	30 --- 44
DISCUSSION	45 --- 55
CONCLUSIONS	56 --- 58
REFERENCES	i --- xii
APPENDIX	
SUMMARY (enclosed separately)	1 --- 4



INTRODUCTION



INTRODUCTION

Until recently anaplasia and related morphological changes were the sole criteria of cellular transformation in cancer. The interpretation of morphological changes is subject to individual variation. Different observers may see different things in the same object. This subjective element in morphological diagnosis is most pronounced in the late stages of metaplasia and dysplasia. This is the source of many diagnostic differences of opinion just at a time where the correct diagnosis is most important from therapeutic and prognostic view point.

Biochemical changes may provide more accurate criteria for recognition of cancerous transformation of the cell, but this advance, however, is in early stages.

The possibility that malignant transformation of the cells may entail a change in the antigenic structure is generally accepted. This change may involve the acquisition of new antigenic substance or may be in the nature of deletion or loss of antigenic component.

Partial or complete loss of blood group isocantigens has been reported for both premalignant and malignant lesions developing from the epithelium in which these substances are normally present.

The presence of blood group isocantigens A, B and O(H) in cells and tissues other than erythrocytes is well documented. They have also been found to be present in various body fluids and glandular secretions. On the basis of presence or absence of these isocantigens in saliva an individual is said to be secretor or nonsecretor. The solubility of A, B, O isocantigens is different in these two groups of individuals. In secretors both alcohol as well as water soluble antigen are present whereas in nonsecretors only alcohol soluble antigens are present.

The distribution of the blood group isocantigens in various tissues of the body is as follows :

- 1. In cell wall of endothelium - through out the cardiovascular system.**
- 2. In cell wall of stratified epithelium - skin, non-keratinizing squamous epithelium and transitional epithelium.**

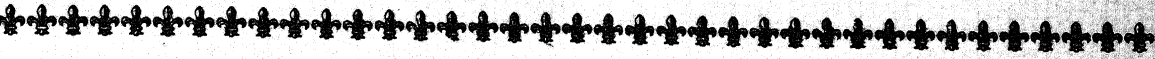
3. In cell wall of simple epithelium + irregular and independent of secretor status.
4. In parenchymal cells and brain tissues + absent.
5. In connective tissue cells + absent.

The A,B,O isoenzymes in tissues can be demonstrated by mixed cell agglutination reaction (MCAR) of specific red cell agglutination (SRCA) reaction, immunofluorescence and immunoperoxidase techniques. MCAR was originally developed to demonstrate the presence of A and B antigens in platelets and epidermal cells. A,B,O groups of the tissues can be reliably determined by this method on paraffin sections.

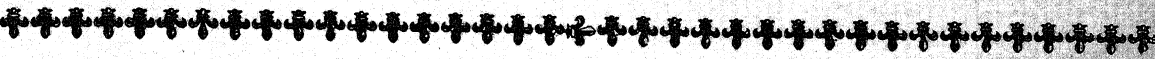
The uterine cervix is commonly the site of development of squamous cell carcinoma. Because of its accessibility the cervix lends itself to the study of the relationship of early lesions to the development of invasive carcinoma. The fate of purely benign reversible lesions such as squamous metaplasia and the more ominous lesions such as severe dysplasia and carcinoma in situ can be studied in details.

4

The importance of early diagnosis of cervical malignancy as regards prognosis and treatment can not be over emphasized. Therefore uterine cervix has especially been selected for the study, with the idea that behaviour of A,B,O isoenzymes may prove to be of considerable diagnostic as well as prognostic value and possibly a guide to therapy.



REVIEW OF LITERATURE



REVIEW OF LITERATURE

Tumor development is a play with many different dramatic persons. The main character is still the cell. It is a protean figure that can appear in many different forms and play different roles, most of which can be classified as stages in microevolutionary process known as tumor progression. The initiation of tumor development is most likely to involve changes at the genetic level. On its road to progression the neoplastic cell encounters many predators, including various effectors of immune system. Inter alia it will depend on host genetics, on age and all of the many physiological modulators of immune system (Klein, 1980).

The structural pattern of malignant tumor cell is sufficiently distinct from the normal cell to be identified in most instances (Sproul, 1956). Subjective differences in their interpretations are always possible, especially in severe dysplasia and intra epithelial neoplasia. The limiting factor in the cure of carcinoma is early diagnosis. Serological methods for diagnosis of malignant tumours (Davidsohn, 1936) are of historical importance and none give promising results.

In the recent past much emphasis has been given on the immunological aspect of neoplasia. The antigenic constitution of the tumor may be different from that of the host. The changes in antigenic constitution may involve the acquisition of new antigenic substance or deletion or loss of antigenic component (Coombs, 1961).

The depletion of specific antigens within tissues has been considered an important factor of neoplastic behaviour, both in experimentally induced tumors in animals and spontaneous tumors in human. A loss of antigenicity of intercellular substance and basement membrane was observed in benign, premalignant and malignant tumors of epidermal origin and it had a parallel course with the grade of cellular anaplasia (Vareltzis et al, 1980). With higher sensitive techniques A B O isoantigens can serve as tracer antigen for the study of changes in malignant transformation.

In the same way as it is impossible to study pathological changes in tissue without a knowledge of normal histology, so it is also necessary to have a full knowledge of the antigenic architecture of normal cell.

The ABO(H) isoenzymes, in addition to their well recognized presence on RBC and secretion of certain individuals, are also expressed in a variety of body tissues.

DISTRIBUTION OF ABO ISOANTIGENS IN VARIOUS TISSUES

It has long been confirmed that ABO isoenzymes are present in cells other than erythrocytes and in secretions other than saliva. They are present in platelets (Gurevitch and Nelken, 1954; Moureau and Ander, 1954), white blood cells (Thomsen, 1930; Dausset, 1956). In mucous secretions (Hartmann, 1941) in epidermal and epithelial cells (Coombs et al, 1956), human oral epithelium (Debelsteen and Fejerskov, 1974) and in spermatozoa (Edwards et al, 1964; Seetchar, 1965). Saulman (1960), Saulman (1964) studied the histological distribution of ABO isoenzymes in humans in intra and extra embryonic life by immunofluorescent (IF) technique in great details. According to him it can be summarized in following six convenient headings (1960) -

- (1) The intima of the vessels of all calibers throughout the body and those supplying malignant tumors contain ABO isoenzymes.

(ii) The stratified epithelia of skin, oral cavity, oesophagus, uterine ectocervix, vagina, Hassall's bodies in thymus and transitional epithelium of lower urinary and upper respiratory passages show that isoeantigens are confined to Malpighian layer in stratified squamous epithelium with a predilection of granular layer in skin and are present in all layers of transitional epithelium.

(iii) The simple epithelia show various degree of completeness of outlining the cell wall wide independent of the secretor status. The parenchymal cells of endocrinal glands and nervous system show absence of antigen.

(iv) The mucous secreting apparatus

In secretors - Salivary glands, lining epithelium of the glands of stomach, goblet cells of small and large intestine up to the level of transverse colon, mucous glands and goblet cells of upper respiratory passage, gall bladder, uterine cervix and pseudomucinous ovarian cyst contain large amount of ABO isoeantigens.

In nonsecretors - ABO isoeantigens are usually not present except in certain special locations like deep parts of gastric foveolae and the

pyloric glands, and varying small number of goblet cells in the crypts of small and large intestine.

(v) Among other organs of secretions and excretions.

The exocrine component of the pancreas, parotid gland, kidney, sweat glands, endometrium, fallopian tube, breast and male genital organs like epididymis, seminal vesicles and prostate secrete ABO isoantigens in secretors. No antigen is found in non secretors.

(vi) In miscellaneous group. The testes show presence of ABO isoantigen in spermatozoa and cells in pro-spermatozoal level. Ova show no antigen and parietal cells of gastric glands are uniformly negative.

The term alcohol soluble and water soluble are for the two varieties of ABO isoantigen. The ethanol resistant water soluble antigens are readily demonstrable in formalin fixed paraffin embedded tissues (Kovarik et al, 1968).

VARIOUS METHODS OF DEMONSTRATION OF ABO ISOANTIGENS

The presence of ABO isoantigens in cells and tissues other than erythrocytes was first demonstrated through the use of an agglutination inhibition test (Kritschewski et al, 1927; Landsteiner, 1926). In

oral mucosa their presence was shown by absorption of isoelectrins in water soluble extracts of oral epithelium (Yosida, 1928).

Immunofluorescent (IF) staining technique (Coons and Kaplan, 1950), mixed cell agglutination reaction (MCAR) (Glyn et al, 1957) and immunoperoxidase (IP) staining technique (Avrameas, 1969) were later used for their demonstration in tissue cells.

Mixed cell agglutination reaction was first described by Topley and Wilson (1935) as quoted by Milgrom et al (1964). Later it was employed in serological testing (Finland and Curves, 1938; Weiner and Harman, 1939), cell suspension (Coombs, 1961), in tissue cultures for the recognition of the species of origin of cell (Coombs et al, 1961), in the ABO grouping of human cells in culture (Hogman, 1960; Chessin et al, 1965) and in studying tissue antigens (Coombs et al, 1956; Cowan, 1962; Milgrom et al, 1964). Tonder et al (1966) used MCAR in frozen sections in order to preserve alcohol soluble antigens. Later Davidsohn and NI (1970) reported that the test could be done on frozen sections of fresh and formalin fixed tissues, on section of recent and old paraffin blocks and on old and new

H&E stained and decolorized slides for reaction.

Moreover age of the sections and of the paraffin blocks do not affect the sensitivity and specificity of the test. Davidsohn and Stajnal (1971) stressed that positive reaction is not the amorphous clumping of agglutination but adherence and for this reason they used the term specific red cell agglutination (SRCA) in place of mixed cell agglutination reaction.

MCAR on formalin fixed paraffin embedded tissue has been reported to be more sensitive than IF technique (Davidsohn et al, 1969), where as Dabalstein and Rygaard (1972) suggest that IF technique seems to be as sensitive as the MCAR but is superior to latter in allowing more accurate localization of the antigens.

It has been shown that the ABO isoenzymes in tissues are not influenced by formalin fixation and paraffin embedding procedures, therefore immunofluorescent staining, immunoperoxidase staining and specific red cell agglutination reaction can be successfully used in sections prepared from formalin fixed paraffin embedded tissues (Kovarik et al, 1968; Dabalstein and Rygaard, 1972). Dorsett and Joachim (1978) have suggested that Bouin's fluid is the better fixative

for immunofluorescent staining studies as in it the antigens and the antibodies are better preserved. Quantitatively ABO (H) isoenzymes differ widely in their concentration in different tissues and quantitative analysis as such is not very much helpful in early diagnosis of malignant lesions (Debelsteen, 1972).

BIOCHEMICAL ASPECTS OF ABO ISOANTIGENS

Biochemically the alcohol soluble and water soluble ABO isoenzymes are glycoproteins and glycolipids respectively and the group specificity is associated with the carbohydrate moiety. The appearance of ABO antigens begin with a precursor mucopolysaccharide substance which is further modulated into H substance and the H substance into AB antigens under genetic control. The genes responsible for this conversion regulate enzyme production for catalysing the transfer of sugar. The L-N-acetyl-D-galactosaminyl and D galactosyl transferases are the enzymes which are necessary for the conversion of H substance with A and B substances respectively (Watkins, 1966). ABO isoenzyme loss may indicate defective biosynthesis (Kuhns, 1978).

The view according to which the isoantigen are derived from H, and may indeed be associated with one and the same mucopolysaccharide gains a further circumstantial confirmation as while in group O, tissue H constitutes the sole antigen of the ABO (H) system, it generally appears also in non O tissue in amount varying from zero to those appearing equivalent to or exceeding A or B (Saulson, 1964).

Alterations of glycosyl transferase enzymes occur frequently in carcinoma tissues in relation to normal adjacent tissues. Schoentag and Kuhns (1970) have reported deficient enzymes in stomach and colon carcinoma. The accumulation of precursor substance, probably due to the block of synthesis of more complex determinants foreign to host, because of the possible activation of allelomorphic genes occurs in human cancers (Young and Nakomori, 1970).

ABO ISOANTIGEN AND CARCINOEMBRYONIC ANTIGEN

Immunochanical studies show that carcinoembryonic antigens (CEA), the tumor markers, are deficient or incomplete ABO blood group antigens and the determinants of blood group antigens and CEA share the same glycoprotein (Alastair et al, 1973; Hausserson et al, 1975).

GENESIS OF ISOANTIGENS IN TISSUES

The problem of origin of the ABO isoantigen on the surface of the epithelial and endothelial cells is complicated by the fact that the absorption of antigen from the surrounding fluid onto the cell surface can be accomplished experimentally. It would seem doubtful whether the concentration of group substance in plasma and tissue fluid is sufficient to be a factor, although in salivary glands and in breast the secreted antigen may contribute to the outlining of the glandular epithelium. The most convincing circumstances arguing for the generally autochthonous character of cell wall antigen, however, is their appearances in the embryo long antedating that of the water soluble forms and their presence in non-secreter locations devoid of the water soluble substances (Szulman, 1964).

CANCEROUS TRANSFORMATION AND ISOANTIGENS

The effect of cancerous transformation^{on} ABO isoantigen is being studied for a long time. The initial studies indicated that ABO isoantigens were not affected by malignant process. Further studies indicate that malignant transformation is essentially associated with antigenic loss (Key, 1957; Kovarik

et al, 1968). ABO isoenzymic status has been studied in tumors of different tissues separately.

Studies on gastrointestinal tract malignancies as a whole (Cowan, 1962; Davidsohn et al, 1966; Rouger et al, 1976), oral malignancies (Dabelsteen and Pindborg, 1973; Dabelsteen et al, 1975; Gupta et al, 1981), stomach malignancies (Denk et al, 1976; Feizi et al, 1979), colon malignancies (Schoentag, 1978; Cooper et al, 1978; Cooper et al, 1979), lung malignancies (Davidsohn and Hi, 1969), nasopharyngeal malignancies (Hawkins et al, 1974), laryngeal malignancies (Lie et al, 1977), ear, nose and throat malignancies (Daysei et al, 1973), breast malignancies (Torti, 1963; Gupta and Schuzin, 1973; Strauchen et al, 1980), skin tumors (England et al, 1979; Nicoles et al, 1980), malignant effusion (Smith et al, 1980), white blood cell cancers (Saichua and Chiewslip, 1978), urinary bladder malignancies (Alroy et al, 1978; Kwon et al, 1979; Linas et al, 1979; Emmott, 1979), prostate malignancies (Gupta et al, 1972), pancreas malignancies (Davidsohn et al, 1971), endometrial malignancies (Gupta, 1976), fallopian tube malignancies (England and Davidsohn, 1973), Uterine cervix malignancies (Davidsohn et al, 1969; Davidsohn et al, 1973; Stafle and Mattingly,

1972; Lill et al, 1976; Bongfiglio and Feindberg et al, 1976) and trophoblastic neoplasms (Mittal et al, 1973) have been carried out. Antigenic loss of varying degree has been found in almost all of them and in many of them it was parallel with the degree of anaplasia and dedifferentiation.

Loss of isoantigen does not occur only in malignancy. It has also been demonstrated in oral mucosa in wound healing, atypia to premalignant lesions (Dabelsteen and Pulling, 1971; Dabelsteen and Fejerskov, 1974; Dabelsteen et al, 1975), in adenomas of parathyroid glands (Woltering et al, 1979), in colon having adenomatous polyps and/or long standing chronic mucosal inflammations (Cooper et al, 1979; Shachan, 1979) in breast having benign proliferative duct lesions associated with fibrocystic diseases (Strauchen, 1980) in urinary bladder mucosa having carcinoma in situ (Weinstein et al, 1979) and in tissue after several passages. In cultures, Hognan (1960) and Chessin (1963) reported that addition of carbohydrate essential for synthesis of A, B & O, to the culture restored ability of the cells to produce antigen.

The vast literature on ABO isoenzymic status of various tissues in normal, neoplastic and nonneoplastic conditions indicate that loss of ABO isoenzyme may serve as an early marker for neoplastic transformation (Davidsohn, 1972; Feizi and Picard, 1978). The uniform expression of ABO isoenzyme by epithelial lining type cells and general absence in mesenchymal connective tissue suggests that ABO isoenzyme expression may be related to epithelial differentiation. Absence of ABO isoenzyme in least differentiated basal layer of stratified squamous epithelium and presence in more differentiated superficial layers support the concept of ABO isoenzyme expression as a marker of differentiated epithelial cell function (Davidsohn et al, 1969). So the loss of normal surface antigen from anaplastic cell may play a significant role in abnormalities of cell recognition such as escape from immune surveillance and loss of contact inhibition (Strauchen et al, 1980). The loss of isoenzyme is not an all or none phenomenon as both positively reacting and negatively reacting cells in H C A R are frequently found in carcinomas. This is probably an evidence of progressive loss in the course of malignant transformation (Davidsohn and Hi, 1970).

In locations like gastrointestinal tract, ovary and epidermis etc. carcinoma is seen as a rule as a fully developed lesion. Only rarely ~~to~~ transition from benign to malignant is encountered.

RELATION OF ANTIGENIC LOSS WITH METASTASIS

It is reasonable to assume that radical change occurs in ^acancer cell before it is released from the tissue and the site of its origin, to grow and multiply at a new location. Any morphologically demonstrable criteria to distinguish the cancer cell that may succeed in overcoming body's defence and form distant metastasis is not yet known. Loss of tissue ABO isoenzyme precedes the formation of distant metastasis in squamous cell carcinoma in uterine cervix, and squamous cell carcinoma, oat cell carcinoma, adenocarcinoma and anaplastic carcinoma of bronchus (Davidson and Ni, 1970) as the loss might be connected with impairment of normal control which limits the cell within the border of the organ of their origin with resultant dissemination of cancer cells and possibility of metastasis (Varehian et al, 1960).

ISOANTIGENS AND CERVICAL NEOPLASIA

The uterine cervix is the common site for the development of squamous cell carcinoma. It was chosen

for first indepth study as the natural history of this squamous cell carcinoma provides an opportunity to follow the transition from benign lesions such as dysplasia to metastatic carcinoma through the stages of carcinoma in situ and invasive carcinoma. The study on the progressive changes in ABO isoenzyme status can easily be carried out on uterine cervix (Davidson, 1969).

A close relationship between the loss of cellular AB and O antigen and malignant transformation in uterine cervix has been shown (Davidson et al, 1969; Davidson et al, 1973; Staff et al, 1973; Bongfigliu et al, 1976). The degree of morphologically demonstrable cellular anaplasia and the decrease or loss of isoenzymes were parallel (Davidson et al, 1970).

In metaplastic and dysplastic epidermis isoenzymes could be demonstrated consistently in amount and distribution compatible with that seen in normal epithelium. In metastatic carcinoma of the uterine cervix isoenzyme could not be demonstrated in primary as well as in metastatic lesion. In the intermediate group including carcinoma in situ and early invasive carcinoma isoenzymes were absent from the cell exhibiting cytological signs of malignancy.

Low density in the distribution of indicator red blood cells and patchy pattern type of reaction in NCAR were explained by the heterogeneity of cellular population of early carcinoma with resulting variation in the ability to produce or to store antigens. It has been suggested that loss of isoantigens is an early indicator of those cellular changes that are the prerequisite for ability to form metastasis (Davidsohn et al, 1969; Davidsohn et al, 1973).

The fate of purely benign and reversible lesions such as metaplasia and more ominous lesions such as severe dysplasia and carcinoma-in-situ of uterine cervix has widely been studied. Dysplasia especially of severe degree is known to lead frequently to invasive carcinoma. Lill et al, (1976) studied the relationship of ABO isoantigens with dysplasia of uterine cervix and demonstrated that loss of ABO isoantigen did not correlate with the morphological grading of dysplasia so was not of significant value in diagnosing dysplastic lesions of cervix.

Marked differences of opinion exist as to the pathological diagnosis of cervical biopsies. In such cases of disputable lesions, as regards their

benignness or malignant behaviour, a demonstrable loss of ABO isoeantigen in tissues by SRCA technique will greatly facilitate interpretations of cervical malignancies. On the other hand the presence of isoeantigens will indicate benignness of the lesion. In SRCA negative cases immunofluorescent technique involving use of labelled FITC will further substantiate the findings of SRCA and the diagnosis of cervical neoplasia.

Morphological examination of cervical dysplasia can not yet predict which lesion will progress to invasive carcinoma and which will regress. This applies also at present time to the immunological methods like SRCA (Davidsohn et al, 1970).

Carcinoma of the uterine cervix is the commonest cancer in females in poor countries like Peru, China and India. The incidence of carcinoma cervix at J.K. Cancer Institute, Kanpur was between 25.4 to 36.7% during 1971-76 and ^{of} all genital malignancies the incidence of invasive carcinoma cervix was found to be 94.5% (Upreti et al, 1981). The use of SRCA on cervical lesions may considerably increase the diagnosis of early stage cancer at which stage complete cure is possible.

M A T E R I A L A N D M E T H O D

For the study 96 examples of cervical lesions from the records of, and the fresh surgically removed specimen received in the Histopathology Section of Pathology Department of M.L.B. Medical College, Jhansi, were selected to represent inflammatory non-neoplastic lesions, dysplasias of mild, moderate and severe degree and invasive carcinoma of well, moderate and poor differentiation. One representative block of each case was chosen out. Out of ninety six cases :

Forty six cases were of invasive carcinoma,

Twenty five cases were of dysplasia and

Twenty five cases were of inflammatory lesions.

Blood group of some of the specimens which were removed by major surgery requiring blood transfusion, were found out from the records of Blood Bank of M.L.B. Medical College Hospital, Jhansi. In cases of fresh specimens blood sample of the patients were taken and the blood grouping was done by slide method. In rest of the cases, blood groups were determined by specific red cell agglutination (SRCA) reaction by treating one of the two sections of the same case by anti-A serum and RBC of group A and another by anti-B

serum and RBC of group B. Positive reaction in either indicates the blood group A or B. Positive reaction in both indicates that the blood group is AB and if none show positive reaction, the blood group is 'O'.

In all the cases, SRCA test was done and in 55 cases immunofluorescence (IF) technique was also applied.

Brief clinical findings, blood group, histological findings and the results of SRCA and IF were recorded on a planned proforma

SPECIFIC RED CELL AGGLUTINATION REACTION (SRCA) :

PRINCIPLE :

The test is based on the three layered sandwich reaction, described by Davidsohn (1971) in which homologous bivalent or polyvalent antisera act as the connecting link between the isoantigen A, B or H present on the tissue as well as on indicator RBC (Fig. No. 1).

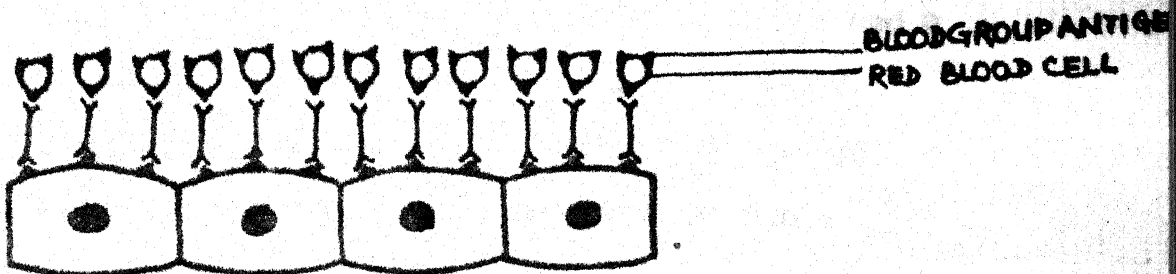
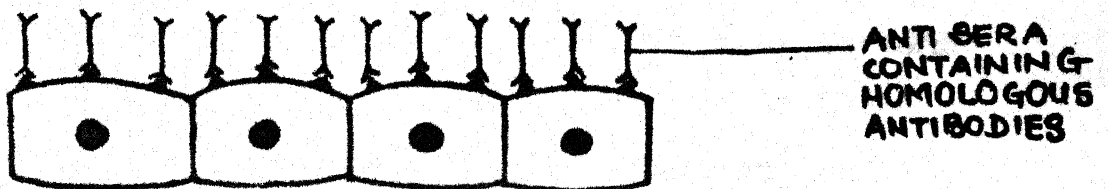
In the three layered sandwich -

the bottom layer - is of tissue,

the middle layer - is of homologous blood grouping antisera and

the top layer - is of homologous indicator red blood cells.

DIAGRAMMATIC REPRESENTATION OF SPECIFIC RED CELL AGGLUTINATION REACTION.



THREE LAYERED SANDWICH TECHNIQUE
(FIGURE NO.1)

MATERIAL :

The following material was used :

Tissue :

Five micron thick sections from each block were mounted on separate microslides smeared with egg albumin. Deparaffinization was done by passing the mounted section through xylene, 90%, 50% alcohol and water for a short duration of time.

Antisera :

Commercially prepared anti A, anti B and anti AB sera with a titre of 512 and anti H sera use with a titre of 256 were used. Anti-A, anti-B and anti-H sera were purchased from Associated Laboratories, Bombay and anti AB serum from Span Diagnostics, Surat.

Indicator red blood cells :

Blood samples belonging to group A, B, AB and O were taken. RBC were washed in three changes of physiological saline and 5% suspension of RBC of each groups were prepared in the same saline.

Physiological saline :

0.9 gm sodium chloride solution in distilled water was prepared in the Chemical Laboratory of Pathology Department of M.L.B. Medical College, Jhansi.

PROCEDURE :

The test was performed in batches of 5-10 cases. Each slide was treated in the following manner :

1. The slide, mounted with tissue section, was placed on a moist filter paper and antiserum was poured on the section and was covered with a petridish for 10 min. at room temperature.
2. The uncombined antiserum remaining on the surface of the section was washed off in three changes of physiological saline each lasting for 10 minutes.
3. The excess saline was drained off and the individual slide was returned to the moist filter paper and covered with 5% suspension of indicator RBC for 10 min. at room temperature. Slide was covered with petridish in order to avoid drying.
4. Another petridish was filled with minimal amount of physiological saline and the slide was turned upside down with a brisk movement and as such placed immediately on the two supporting wooden sticks in the saline filled petridish so that it just touched the saline.
5. After a few min. the slide was shifted over a clear area and after allowing 20-30 min. for indicator RBC that did not react specifically with the antiserum,

to become detached and sink to the bottom of petridish slide was finally moved aside on a clear area.

6. The slide still remaining in the petridish was then examined with low power of microscope through the thickness of the slide with tissue section remaining on the lower surface using 5X and 15X eye pieces.

CONTROL :

To ensure that the SRCA reaction were specific, the following controls were applied :

A. Tissue control :

I. Intrinsic positive control -

1. Endothelial lining of blood vessels.
2. RBC present in the section.
3. Epithelial cells of normal tissue adjacent to lesion.

II. Intrinsic negative control - connective tissue.

B. Reagent control :

1. Heterologous antisera and homologous RBC were used e.g. In group A section anti-B serum and group A RBC were used.

2. Homologous antisera and heterologous RBC were used e.g. in group A section anti-A serum and group B RBC were used.

3. Blood grouping by NCAR reaction also served as a reagent control.

INTERPRETATIONS :

'-' Negative - no adhesion.

'±' Doubtful positive - the result patchy with some areas show clear adhesions while other areas show no adhesion. Also included in this group are sections that show adhesion only in lower or top third of epithelium.

'+' Weak positive - all the cells do not show adhesion but weakly positive. Adhesion is diffuse not patchy.

'++' Moderately positive almost all cells show adhesion.

'+++' Strongly positive - over crowding of adhered red blood cells.

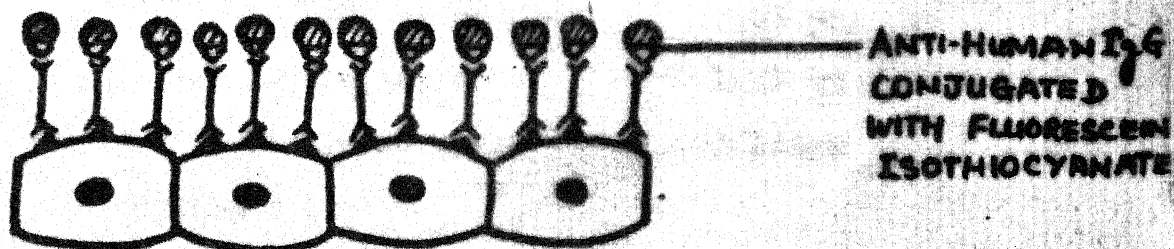
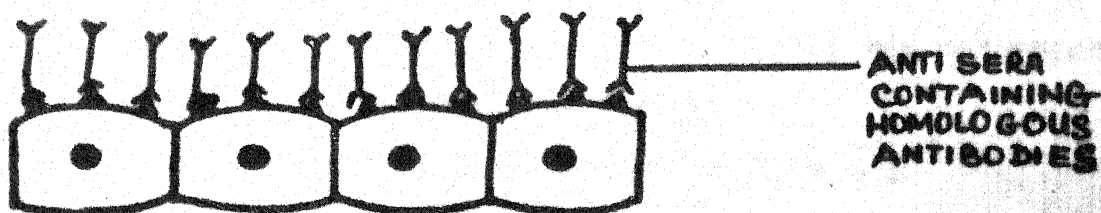
IMMUNOFLUORESCENCE STAINING TECHNIQUE :

PRINCIPLE :

Immunofluorescence (IF) staining technique is based upon a double layer fluorescence staining method used on sections cut from formalin fixed paraffin embedded tissue as described by Coons and Kaplan (1950) (Fig. No. 2).

In the double layer fluorescence staining the first layer - is of homologous blood grouping

DIAGRAMMATIC REPRESENTATION OF IMMUNOFLOUORESCENCE STAINING



DOUBLE LAYER STAINING TECHNIQUE

antisera and
the second layer - is of antihuman IgG (Goat),
conjugated with fluorescein
isothiocyanate (FITC).

MATERIAL :

Tissue :

Same as in SRCA reaction.

Antisera :

Anti-A, anti-B and anti-AB antisera as used in
SRCA reaction.

Conjugate :

Commercially prepared Goat antihuman IgG conjugated
with fluorescein isothiocyanate (FITC) was purchased
from DECRUZ CORPORATION, Bombay.

Phosphate Buffer saline (PBS) of pH 7.1 :

Was prepared in the Chemical Laboratory of the
Pathology Department of M.L.B. Medical College,
Jhansi, using the following formula :

NaCl	8.50 gm.
Na_2HPO_4 (anhydrous)	1.07 gm.
$\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$	0.39 gm.
Distilled water	1 litre

Mountant :

Glycerol and PBS in equal parts.

PROCEDURE :

1. Slides were incubated with appropriate antisera in
a moist chamber at room temperature for 30 minutes.

2. Slides were washed in three changes of PBS, each lasting for 5 minutes.
3. Slides were incubated for further 20 minutes with FITC (in 1:4 dilution in PBS).
4. Slides were washed again in three changes of PBS, each lasting for 5 minutes.
5. Slides were mounted in glycerol mountant and studied by fluorescence microscope.

A "Leitz" Fluorescence microscope fitted for incidental illumination with Ploemopak 2 (quick-change exciter mirror filter turret) in the microscope tube was used. The light source was 200 W/4 ultra high pressure Mercury lamp. Immersion type objectives (10X and 25X) and low power oculars (4X) with built in filter for protecting the eyes, were used. Primary filter was FITC interference blue filter.

CONTROL :

- A. Tissue control: same as in SRCA reaction.
- B. Reagent control: Heterologous antisera was used which served as negative control.

INTERPRETATIONS :

- '+' Positive - showing apple green fluorescence.
 - '-' Negative - showing no fluorescence.
-



OBSERVATIONS



OBSERVATIONS

The present work embodies the study of 96 cases of carcinoma, dysplasia and inflammatory lesions of uterine cervix. Sections for the study were prepared from old (75) and fresh (21) paraffin blocks of cervical biopsy specimen received in the Department of Pathology, M.L.B. Medical College, Jhansi.

Table - I

SHOWING THE TOTAL NUMBER OF CASES OF DIFFERENT CERVICAL LESIONS STUDIED BY H&E AND IF STAINING

Sl. No.	Cervical lesion	No. of cases studied	
		By H&E	By IF staining
1.	Squamous cell carcinoma	46	30
	Microinvasive carcinoma	1	1
	Well differentiated carcinoma	6	4
	Moderately differentiated carcinoma	15	10
	Poorly differentiated carcinoma	24	15
2.	Dysplasia	25	15
	Mild dysplasia	12	7
	Moderate dysplasia	8	5
	Severe dysplasia	5	3
3.	Chronic cervicitis	25	10

Table No. 1 reveals that out of 96 cases, 46 cases were of invasive squamous cell carcinoma and all

were studied by mixed cell agglutination reaction (MCAR) and 30 were studied by immunofluorescence (IF) staining also. The 46 cases included 1 case of microinvasive carcinoma, 6 cases of well differentiated carcinoma, 15 cases of moderately differentiated carcinoma and 24 cases of poorly differentiated carcinoma. Out of 30 cases of carcinoma studied by IF, 1, 4, 10 and 15 cases were of microinvasive, well differentiated, moderately differentiated and poorly differentiated carcinoma respectively.

Out of 25 cases of dysplasias studied by MCAR 15 cases were studied by I.F. staining also. In MCAR study 12 cases were of mild, 8 of moderate and 5 were of severe dysplasia. IF staining was done in 7 cases of mild, 5 cases of moderate and 3 cases of severe dysplasia.

Twentyfive cases in MCAR and 10 cases in IF staining of chronic cervicitis were included to serve as a control group of cases.

Table No. 2 shows the age distribution of total 96 cases of cervical biopsies studied. Out of 46 cases, maximum number of cases were of 40 to 44 years of age (26.1%) & 69.6% of the cases were encountered within 35 to 49 years of age.

Table - II
AGE GROUP VERSUS CERVICAL LESIONS

Age group (in years)	No. of cases (%)		
	Carcinoma	Dysplasia	Chronic cervicitis
20 - 24	- -	- -	1 (4.0)
25 - 29	4 (8.6)	1 (4.0)	- -
30 - 34	4 (8.6)	1 (4.0)	1 (4.0)
35 - 39	9 (19.6)	9 (36.0)	4 (16.0)
40 - 44	12 (26.1)	4 (16.0)	7 (28.0)
45 - 49	11 (23.9)	5 (20.0)	8 (32.0)
50 - 54	3 (6.6)	2 (8.0)	3 (12.0)
55 - 59	2 (4.4)	1 (4.0)	- -
60 - 64	1 (2.2)	2 (8.0)	- -
65 - 69	- -	- -	- -
70 - 74	- -	- -	1 (4.0)
75 - 79	- -	- -	- -
	46 (100.0)	25 (100.0)	25 (100.0)

Maximum number of the cases of cervical dysplasia were of 35 - 39 years of age (36.0%), and 72.0% cases were within 35 - 49 years of age.

Thirty two percent cases of chronic cervicitis were of 45 - 49 years of age, 76% of the cases were within 35 - 49 years of age. However, one case was observed in 20 - 24 years and 70 - 74 years of age groups.

Table - III

SHOWING RELATIONSHIP OF BLOOD GROUPS TO
DIFFERENT CERVICAL LESION

Cervical lesion	Total No. of cases	No. of cases of different blood groups (%)			
		A	B	AB	O
Squamous cell carcinoma	46	10 (21.73)	22 (47.82)	2 (4.34)	12 (26.10)
Dysplasia	25	4 (16.0)	11 (44.0)	1 (4.0)	9 (36.0)
Chronic cervicitis	25	8 (32.0)	7 (28.0)	5 (20.0)	5 (20.0)
Control	1271	275 (21.64)	466 (36.67)	100 (7.87)	430 (33.83)
Statistical significance*					
Carcinoma Vs control	t=	0.01	1.56	0.89	1.10
	P>	0.90	0.10	0.30	0.20
Dysplasia Vs control	t=	0.68	0.75	0.71	0.23
	P>	0.40	0.40	0.40	0.80

* $P > 0.05$ Insignificant

Table No. 3 shows the relationship of blood groups to inflammatory, dysplastic and malignant lesions of uterine cervix as compared to the distribution of ABO blood groups in 1271 apparently normal subjects.

No statistically significant relation in distribution of ABO blood groups in cases of cervical dysplasia and carcinoma was found.

MCAR IN CERVICAL LESIONS

The isoantigens were studied by MCAR and results were recorded, depending upon the agglutination of red blood cell in the lesional area.

1. '++' Strongly positive - indicating normal isoantigenic status.
2. '+' Weakly positive - indicating partial loss of isoantigen.
3. '±' Equivocal - indicating partial or complete loss of isoantigens.
4. '-' Negative - indicating complete loss of isoantigen.

Table - IV
SHOWING MCAR FINDINGS IN CASES OF INFLAMMATORY
LESIONS OF UTERINE CERVIX

Lesion	Total No. of cases	No. of cases showing different reaction (%)			
		++	+	±	-
Chronic cervicitis	25	21 (84.0)	4 (16.0)	-	-

As is clear from the table No. IV strongly positive MCAR was observed in 21 (84%) cases of chronic cervicitis. The reaction was weakly positive in 4 (16%) cases (Fig. No. 3).

Table - V
SHOWING NCAR FINDINGS IN CASES OF DYSPLASTIC
LESIONS OF UTERINE CERVIX

Lesion	Total No. of cases	No. of cases showing different reaction (%)			
		++	+	±	-
Dysplasia	25	5 (20.0)	9 (36.0)	9 (36.0)	2 (8.0)
Mild dysplasia	12	3 (25.0)	5 (41.7)	4 (33.3)	-
Moderate dysplasia	8	2 (25.0)	2 (25.0)	3 (37.5)	1 (12.5)
Severe dysplasia	5	-	2 (40.0)	2 (40.0)	1 (20.0)

Thus, out of 12 cases of mild dysplasia 5 cases (41.7%) showed weakly positive (+) NCAR. Reaction was strongly positive (++) in 3 cases (25.0%) and equivocal in 4 cases (33.3%) of mild dysplasia (Fig. No. 4).

Out of 8 cases of moderate dysplastic lesion of uterine cervix, isocantigens were found to be completely lost in 1 case (12.5%). Isocantigens were variably lost, as indicated by ± NCAR in 3 cases (37.5%). Reaction was weakly positive (+) or strongly positive (++) in rest of the 50% cases (Fig. No. 5).

As regards NCAR in severe dysplasia of cervix, the study included only 5 cases of severe dysplasia

and only one case (20%) showed complete antigenic loss as indicated by negative NCAR. Results of NCAR were equivocal in 2 cases (40%) of severe dysplasia of uterine cervix. Reaction was weakly positive in 2 cases (40%) indicative of partial loss of isantigens from the tissues (Fig. No.6).

Table - VI
SHOWING NCAR FINDINGS IN CASES OF CARCINOMA
OF UTERINE CERVIX

Lesions	Total No. of cases	No. of cases showing different reaction (%)			
		++	+	±	-
Carcinoma	46	1 (2.2)	5 (10.8)	8 (17.4)	32 (69.6)
Microinvasive carcinoma	1	-	-	1 (100)	-
Well differentiated carcinoma	6	-	1 (16.7)	1 (16.7)	4 (66.6)
Moderately differentiated carcinoma	15	-	2 (13.3)	4 (26.7)	9 (60.0)
Poorly differentiated carcinoma	24	1 (4.2)	2 (8.3)	2 (8.3)	19 (79.2)

In cases of squamous cell carcinoma of the uterine cervix, NCAR findings varied from strong positive (++) to negative (-) (Table VI). In one case of microinvasive carcinoma, NCAR was equivocal.

Table - VII

IMMUNOFLUORESCENCE (IF) STAINING RESULTS IN CHRONIC CERVICITIS

Lesion	Total No. of cases	No. of cases showing different reactions (%)	
		+	-
Chronic cervicitis	10	9 (90.0)	1 (10.0)

As is apparent from the table No. VII, out of 10 cases of chronic cervicitis, 9 cases (90.0%) showed positive reaction and only one case (10.0%) showed negative reaction, indicating absence of demonstrable iscontigen.

Table - VIII

IF STAINING RESULTS IN DYSPLASIA OF UTERINE CERVIX

Lesion	Total No. of cases	No. of cases showing different reactions (%)	
		+	-
Dysplasia	15	10 (66.7)	5 (33.3)
(a) Mild dysplasia	7	6 (85.7)	1 (14.3)
(b) Moderate dysplasia	5	3 (60.0)	2 (40.0)
(c) Severe dysplasia	3	1 (33.3)	2 (66.7)

Out of 15 cases of dysplasia of uterine cervix 10 (66.7%) showed positive and 5 (33.3%) showed negative

Well differentiated carcinoma showed complete loss of isocytogen in 4 (66.6%) cases and partial loss in 1 (16.7%), cases, whereas 1 case (16.7%) gave equivocal results. In cases of moderately differentiated carcinoma majority (9 cases, 60.0%) was of the cases showing negative reaction, 4 cases (26.7%) showed equivocal reaction and 2 cases (13.3%) showed partial loss of isocytogens. Out of 24 cases of poorly differentiated carcinoma studied, 19 cases (79.2%) showed complete loss of isocytogens as demonstrated by negative MCAR. Results of MCAR were weakly positive in 2 cases (8.3%) and equivocal in another 2 cases (8.3%). It was only in one case (4.2%) that the MCAR findings were suggestive of presence of isocytogens in tissues without any demonstrable loss (Fig. No. 7, 8, 9 & 10).

IMMUNOFLUORESCENCE STAINING IN CERVICAL LESIONS :

To substantiate the results of MCAR, isocytogens in tissues were studied also by immunofluorescence (IF) staining technique. Depending upon the staining of the tissue at the site of lesion results of IF staining were recorded as :

1. '+' Positive - indicating presence of isocytogen.
2. '-' Negative - indicating absence of isocytogen.

reaction. Among the 7 cases of mild dysplasia positive staining was demonstrated in 6 (85.7%) cases and negative in 1 (14.3%) case. On the contrary 66.7% cases of severe dysplasia showed negative reaction while 1 case showed positive reaction. In cases of moderate dysplasia, number of cases showing positive and negative reaction were 3 (60.0%) and 2 (40.0%) respectively.

Table - IX
IF STAINING RESULTS IN CARCINOMA OF UTERINE CERVIX

Lesion	Total No. of cases	No. of cases showing different reactions (%)	
		+	-
Squamous cell carcinoma	30	2 (6.7)	28 (93.3)
(a) Microinvasive carcinoma	1	-	1 (100.0)
(b) Well differentiated carcinoma	4	-	4 (100.0)
(c) Moderately differentiated carcinoma	10	1 (10.0)	9 (90.0)
(d) Poorly differentiated carcinoma	15	1 (6.7)	14 (93.3)

Table IX shows IF staining result in carcinoma cervix. Total number of cases of carcinoma studied by IF staining were 30 and out of these, results were

negative in 28, (93.3%) cases and positive in 2 (6.7%) cases. All the cases of microinvasive and well differentiated carcinoma of cervix showed negative results. In cases of moderately differentiated carcinoma out of 10 cases, 9 cases (90.0%) showed a negative IF staining whereas only 1 case (10.0%) showed positive staining. Same was the case with poorly differentiated carcinoma in which also 1 case (6.7%) showed positive staining. Fourteen out of 15 cases of poorly differentiated carcinomas showed negative result.

Table - X

HCAR VERSUS IF STAINING FINDINGS IN CHRONIC CERVICITIS

Reaction	HCAR	IF staining	IF
	No. of cases		No. of cases
++	8	+	8
	--	-	--
+	2	+	1
	--	-	1
±	--	+	--
	--	-	--
-	--	+	--
		-	--

As per table X eight cases showed '++' in HCAR and '+' reaction in IF staining whereas out of 2 cases

showing '+' reaction in NCAR, one case showed positive and another showed negative IF staining.

Table - XI

NCAR VERSUS IF STAINING FINDINGS IN CERVICAL DYSPLASIA

Lesion	NCAR Reaction	No. of cases	IF Reaction	No. of cases
Mild dysplasia (total Num- ber of cases 7)	++	1	+	1
			-	--
	+	4	+	4
			-	--
	±	2	+	1
			-	1
	-	--	+	--
Moderate dysplasia (Total number of cases 5)			-	--
	++	1	+	1
			-	--
	+	1	+	1
			-	--
	±	3	+	--
			-	3
Severe dysplasia (Total number of cases 3)			+	--
			-	--
	+	1	+	1
			-	--
	±	1	+	--
			-	1
	-	1	+	--
			-	1

Table - XII
MCAR VERSUS IF STAINING FINDINGS IN CARCINOMA
OF UTERINE CERVIX

Lesion	MCAR		IF	
	staining	No. of cases	staining	No. of cases
Microinvasive carcinoma (Total number of cases 1)	++	--	+	--
			-	--
	+	--	+	--
			-	--
	±	1	+	1
			-	1
	-	--	+	--
			-	--
Well differentiated carcinoma (Total number of cases 4)	++	--	+	--
			-	--
	+	1	+	1
			-	1
	±	1	+	1
			-	1
	-	2	+	1
			-	1
Moderately differentiated carcinoma (Total number of cases 10)	++	-	+	--
			-	--
	+	2	+	1
			-	2
	±	2	+	2
			-	2
	-	6	+	1
			-	5
Poorly differentiated carcinoma (Total number of cases 15)	++	1	+	1
			-	1
	+	2	+	1
			-	2
	±	2	+	1
			-	2
	-	10	+	1
			-	10

Table XI compares the NCAR finding with IF reaction in cases of cervical dysplasia. Cases of dysplasia showing strong positive (++) or weak positive (+) NCAR gave positive results in IF staining also and negative cases of NCAR were negative in IF also. Three cases of moderate and 1 case of severe dysplasia giving equivocal (+) NCAR showed negative IF staining whereas out of 2 cases of mild dysplasia showing equivocal NCAR, 1 case showed positive and another case showed negative IF staining.

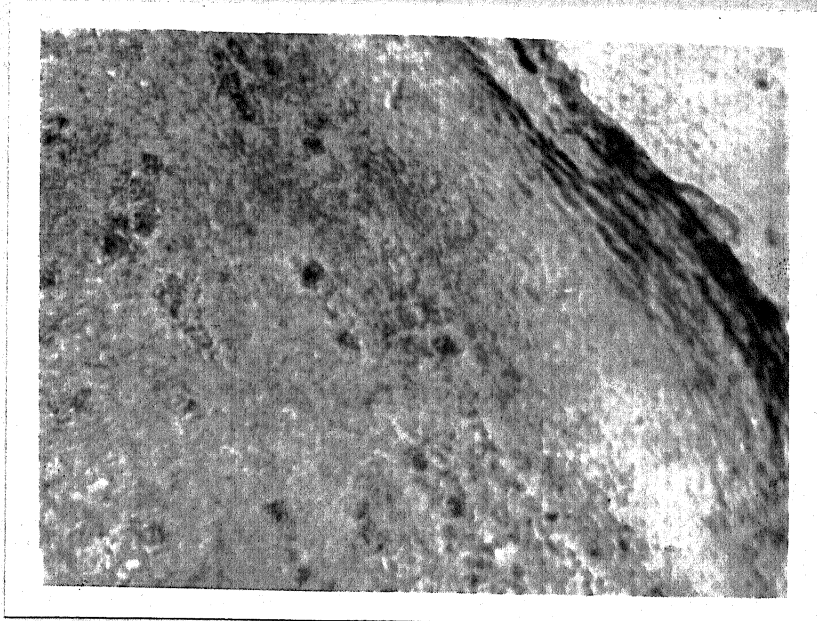
Table No. XII shows NCAR findings versus IF reaction in cases of carcinoma of uterine cervix. Thus in 1 case of microinvasive carcinoma IF staining showed negative results whereas NCAR findings were equivocal in the same case.

In cases of well differentiated carcinoma, IF reactions were negative whereas, NCAR findings were negative in two cases and weakly positive and equivocal in one case each.

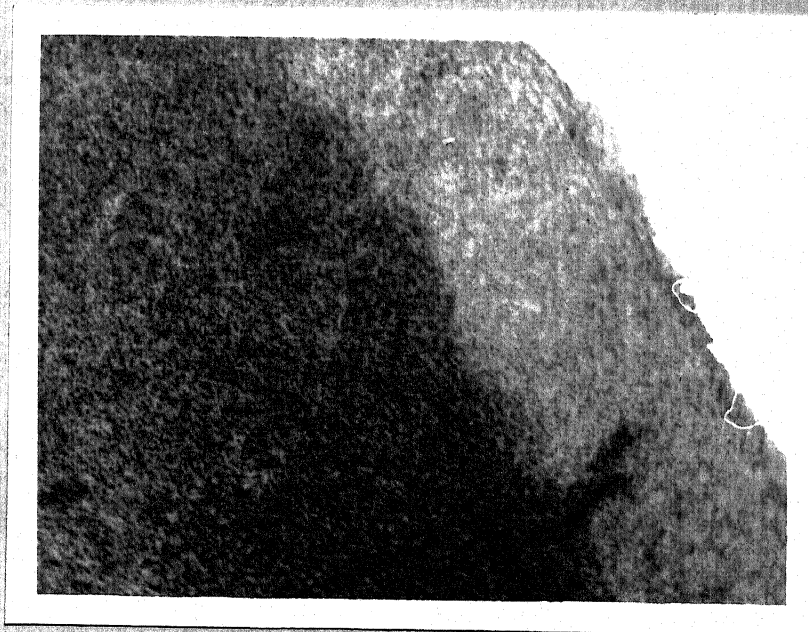
Out of 10 cases of moderately differentiated carcinoma, 6 cases showing negative result in NCAR and 2 cases having equivocal (+) NCAR results, showed negative results in IF whereas out of 2 cases which showed weakly positive (+) results in NCAR .

IF staining results were positive in one case and negative in another case.

In cases of poorly differentiated carcinoma out of 15 cases, 14 cases showed negative IF staining whereas out of these 14 cases, MCAR findings were negative in 10 cases, equivocal in 2 cases and weakly positive in rest of the 2 cases. One case showing strongly positive (++) MCAR showed positive (+) reaction in IF staining.



A



B

Fig. 3 (A) - Normal ectocervix, (H&E:70X)
(B) - Same section showing strongly
positive HCAR, (70X).

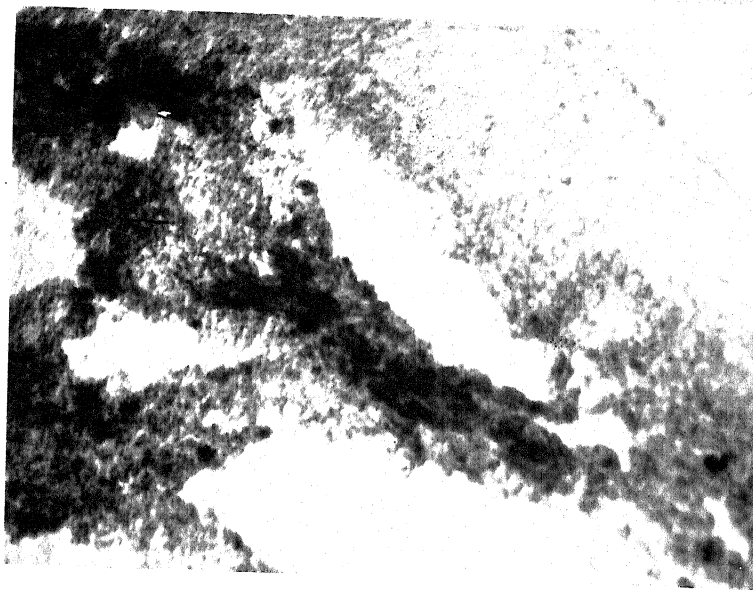
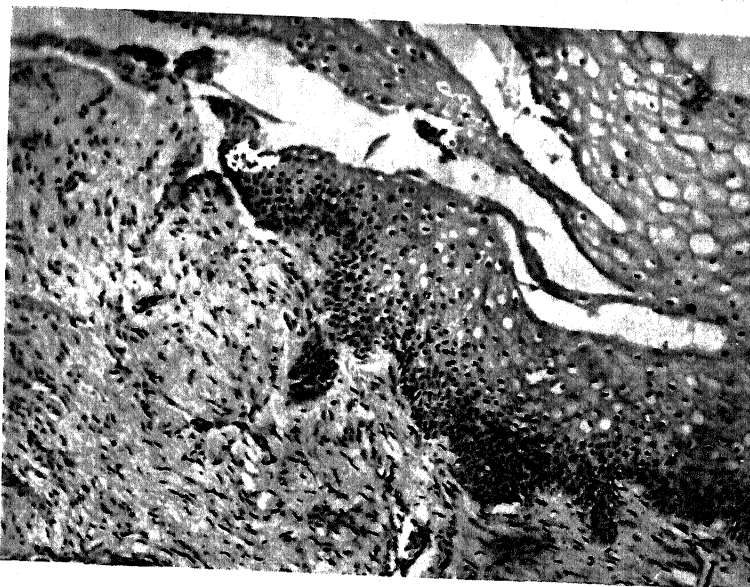


Fig. 4 (A) - Mild dysplasia of uterine cervix. (H&E:70X).

(B) - Same section showing weakly positive MCAR in lesional area. (70X).



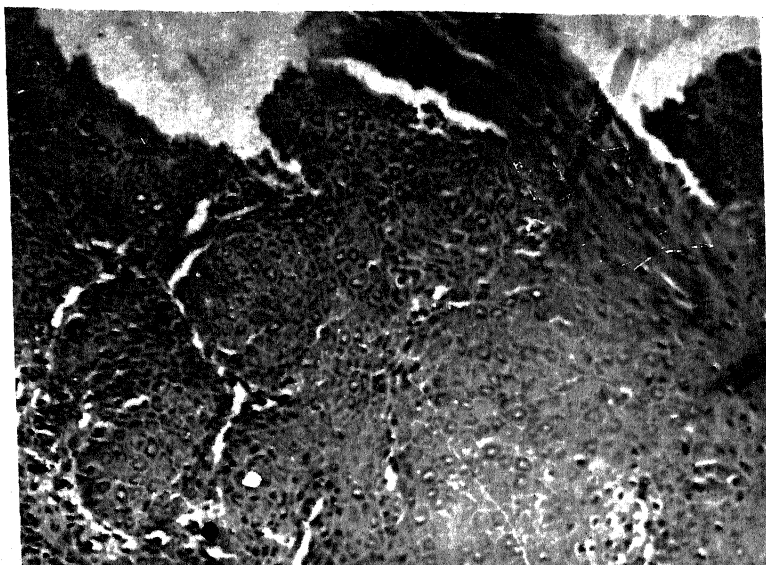
A



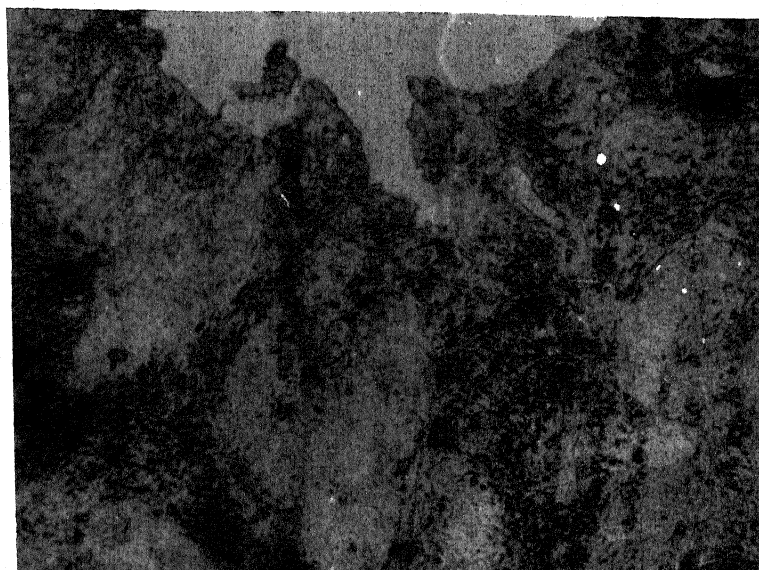
B

Fig. 5 (A) - Moderate dysplasia of uterine cervix, (H&E:70X).

(B) - Same section showing equivocal MICAR, (70X).



A



B

Fig. 7 (A) - Microinvasive squamous cell carcinoma of uterine cervix. (H&E: 70X).

(B) - Same section showing equivocal MCAR. (70X).

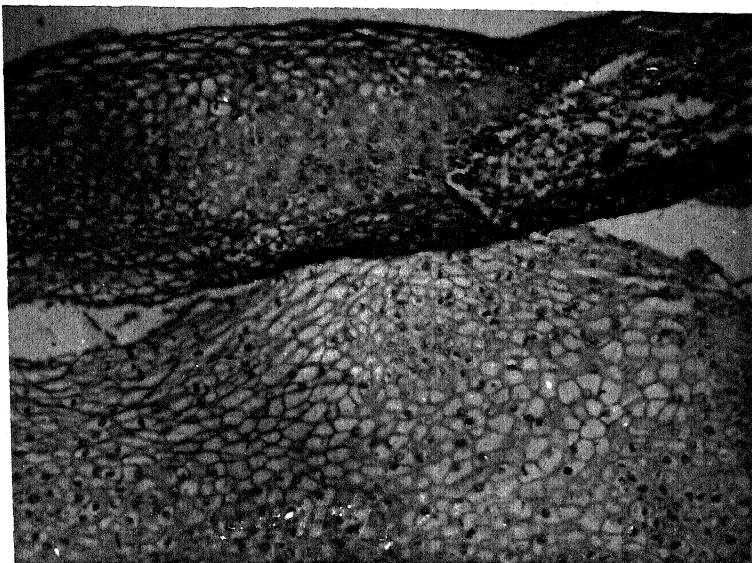


Fig. 6 (A) - Severe dysplasia of uterine cervix, (H&E:70X),
 (B) - Same section showing weakly positive HCAR in lesional area, (70X).

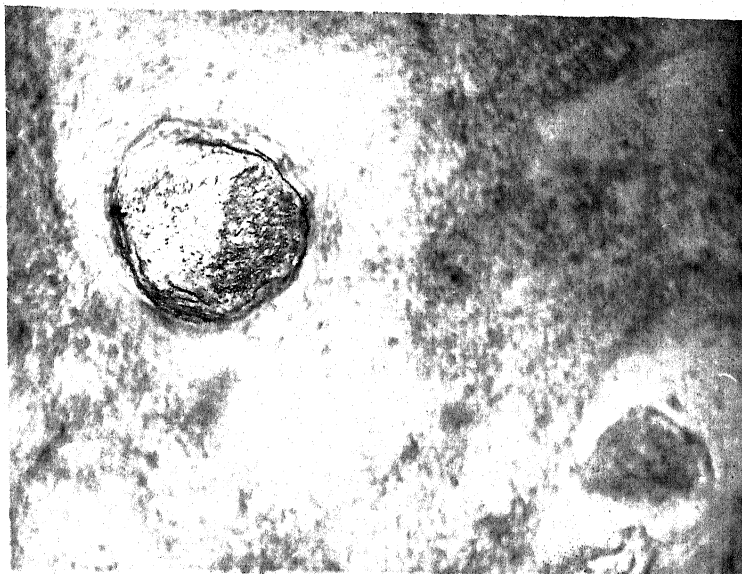
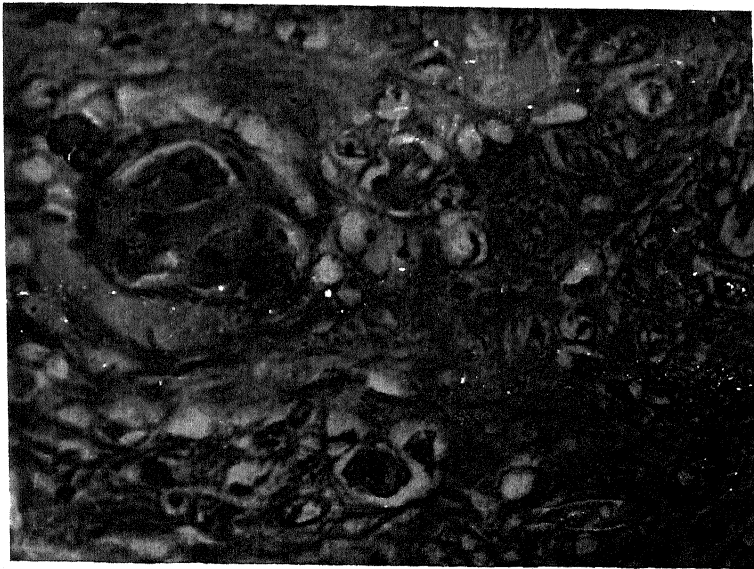


Fig. 8 (A) - Well differentiated squamous cell carcinoma of uterine cervix. (H&E:70X).

(B) - Same section showing negative PCAR. A few RBC(s) adherent to the centre of the epithelial pearl. (70X).

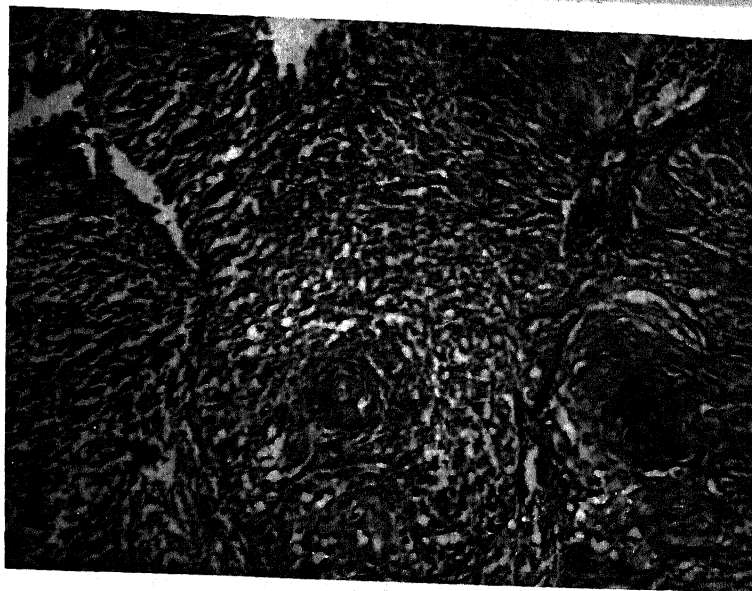


Fig. 9 (A) - Moderately differentiated squamous cell carcinoma of uterine cervix. (H&E:70X).
 (B) - Same section showing equivocal MCAR in lesional area. (70X).

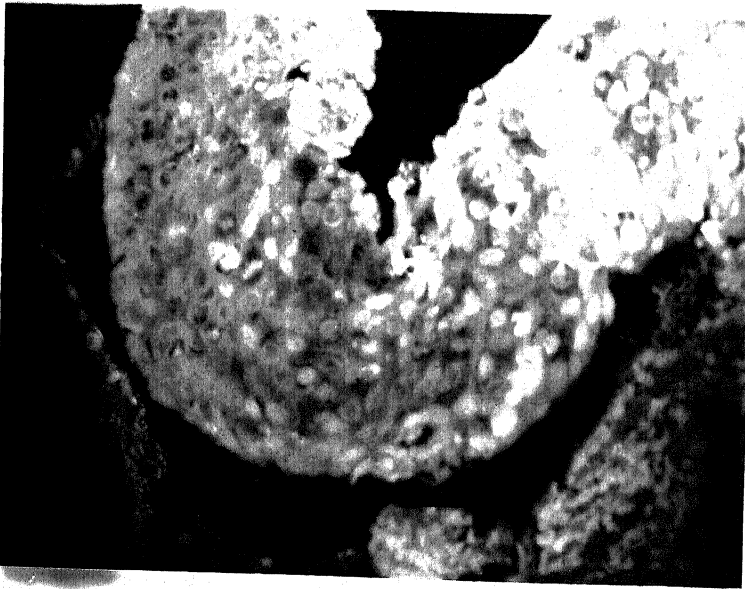


Fig.11 -

Ectocervix showing severe dysplasia with areas of loss of antigen as revealed by IF technique. X100

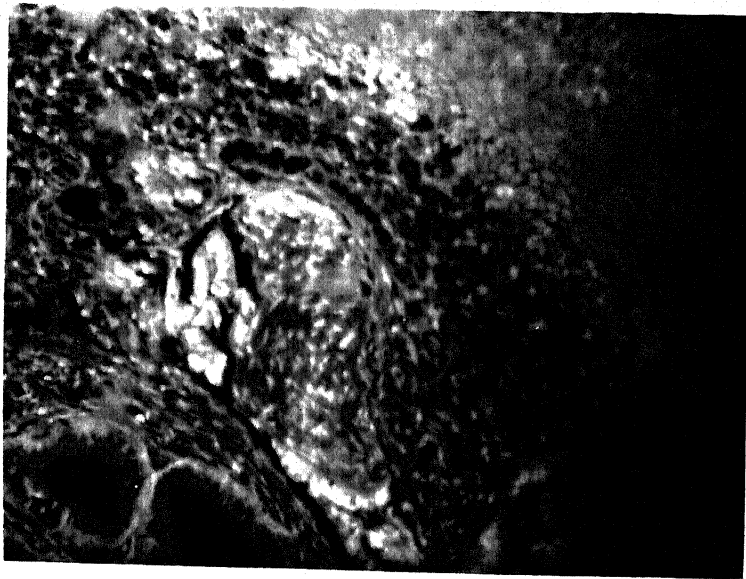


Fig.10 -

Mild dysplasia of ectocervix. No antigenic loss as revealed by IF technique. X100

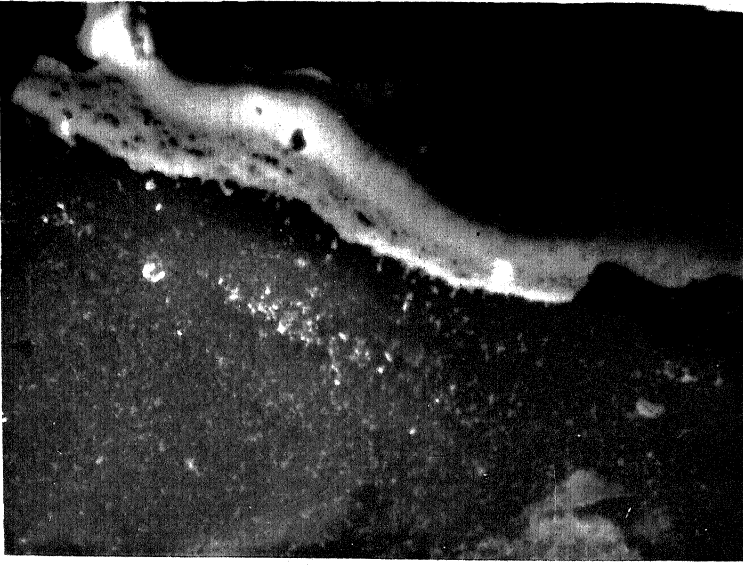


Fig.9 -

Normal ectocervix. No antigenic loss as revealed by IF technique. X100

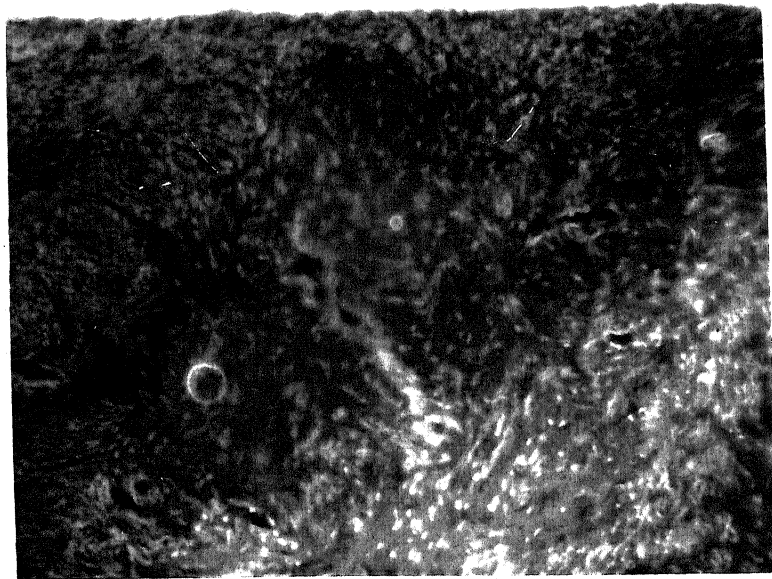


Fig. 14 -

Cervix showing poorly differentiated squamous cell carcinoma with loss of antigen in lesional area as revealed by IF technique. X100

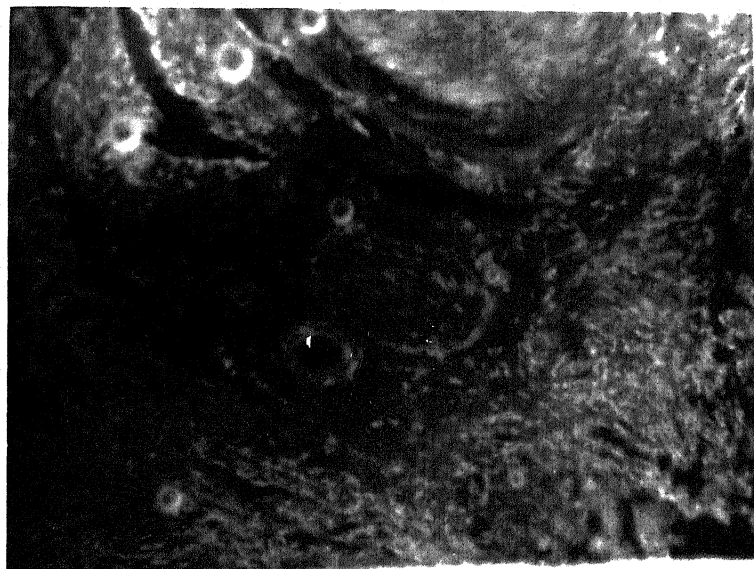


Fig. 13 -

Cervix showing moderately differentiated squamous cell carcinoma with loss of antigen in lesional area as revealed by IF technique. X100

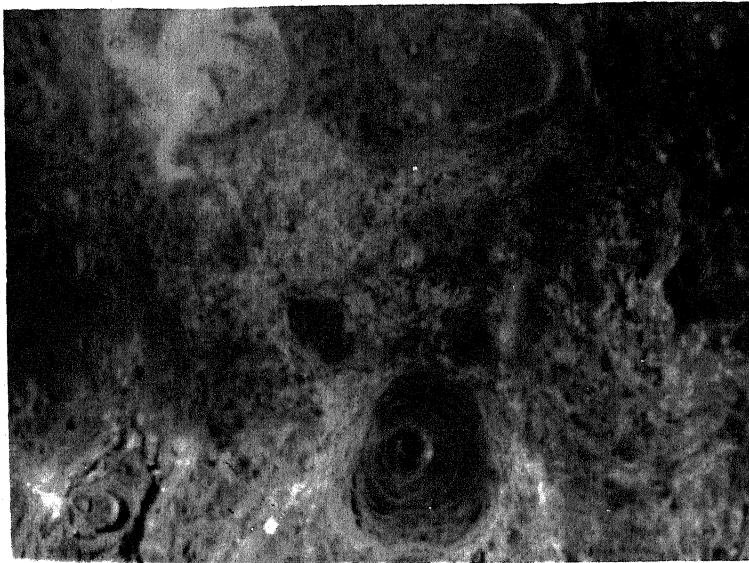


Fig. 12 -

Cervix showing well differentiated squamous cell carcinoma with loss of antigen in the centre of the epithelial pearls as revealed by IF technique. X100

DISCUSSION

Carcinoma of the uterine cervix is the most common cancer in females in poor countries like India. It accounts for 55-60% of genital tract cancers in the females. The disease presents a great challenge to the pathologist. It has been estimated that 2 of every 100 females are prone to develop cervical cancer by the age of 40 years.

Unfortunately the disease commences in a very insidious fashion, and is often well advanced before a correct diagnosis is made, hence great stress is laid, at the present time on early diagnosis of cervical neoplasia.

While early detection continues to be the best weapon against cancer mortality, controversy exists in differentiating microscopically the benign cellular aberrations from their malignant counterparts.

The human uterine cervix is unique in that it is a site of an epithelial neoplasia, occurring with a high prevalence rate and is accessible, easily detectable and amenable to long term study with little discomfort to the patient.

With the emergence of immunological aspect of neoplasia, attention was drawn towards the ABO

isocitogen expressibility of neoplastic cell. Using mixed cell agglutination reaction (MCAR), the antigenic behaviour of the neoplastic cell in neoplasias of various tissues and organs has been studied by a large number of workers and deficient expression has been found in most of the cases as compared to the normal nonneoplastic cells (Cowan, 1962, Davidson et al, 1969, Debeltsen, 1973, Benk, 1976, Schoentag, 1976, Stretcher et al, 1980). Loss of isocitogen has also been reported to occur in dysplasias, cellular atypia and during healing processes (Debeltsen et al, 1974, Weinstein et al, 1978, Molterling et al, 1979, Cooper et al, 1979). More recently other new and sophisticated techniques like immunofluorescent (IF) and immunoperoxidase (IP) staining have also been tried to demonstrate the isocitogens in tissues with an idea to find out if these are superior to MCAR.

The present study was undertaken to investigate the relationship between the histopathological diagnosis of dysplasia and carcinoma of uterine cervix and the presence or absence of isocitogens in these lesions, in order to find out if it may help to determine the likelihood of established or imminent carcinoma of the uterine cervix.

The study is based on the observations recorded in ninety six cases of cervical lesions. Out of these 96 cases of cervical lesions, 46 cases were of invasive squamous cell carcinoma including one case of ^{micro} invasive carcinoma, 25 cases were of dysplasia and 25 cases were of chronic cervicitis which served as control group for the study. Present study was chiefly directed towards the demonstration of isoenzymes in cervical neoplasia by using NCAR and IF techniques. All the observations were recorded in the form of tables as shown in the previous chapter.

The peak incidence of cervical carcinoma was found to be at the age of 35-49 years (69.7%). Upreti et al (1981) in their series reported the peak incidence of cervical carcinoma at the age of 40-49 years (66.7%). Further in the present study the cases of dysplasia were mostly in 35-49 years of age (72.0%). Other workers have reported the peak incidence (90.4%) of dysplasia of uterine cervix in 30-49 years of age (Upreti et al, 1981).

The results of the present study do not show any significant preponderance of any of ABO blood groups in patients with carcinoma or dysplasias of cervix.

These findings are different from the observations of Amiani and Shojwani (1981) who found a statistically significant higher incidence of carcinoma of uterine cervix in blood group 'B' patients (45.0%) as compared to the incidence of blood group 'B' (36.6%) in the control group in their study.

The difference in these results may be due to the small number of cases studied. For proper evaluation of association of ABO blood groups with carcinoma cervix, it is necessary that a larger number of cases be analysed.

1. NEAR FINDINGS IN CERVICAL LESIONS

(Fig.no.11).

1. In chronic cervicitis :

Isocantigens were found to be present in all the layers of covering epithelium of the portiovaginalis of cervix (excluding basal layer), endo cervical glands and the endothelial lining of blood vessels as disclosed by uniformly closely packed adhesion of red blood cells to the tissues mentioned above in 21 cases (84.0%) out of total 25 cases of chronic cervicitis studied.

Uniformly sparse adhesion indicated in the form of '+' reaction was observed in rest of the cases. Many other workers have demonstrated normal antigenic status in all the cases of chronic cervicitis studied (Kovarik et al, 1968, Davidson et al, 1968, Bongfiglio, Feindberg,

1976). However, Lill et al (1976) in their study of cases of chronic cervicitis, have reported partial and complete loss of isocntigens (16.1% and 41.7% respectively). Similar partial loss of isocntigen as indicated by weaker NCAR in small number of cases (16.1%) in chronic cervicitis in the present study might be due to some functional changes in the epithelium accompanying dysplasia predating morphological changes. It is quite probable that biopsies from the neighbouring areas could have shown morphologically demonstratable dysplastic changes in the epithelium.

2. In cervical dysplasia.

In the present study NCAR disclosed a variability of antigenic expression, ranging from no loss (++) reaction) in 20% cases, to complete loss (- reaction) in 8% cases. Whereas, in the study of Davidsohn and Ni (1970) none of the case of cervical dysplasia showed loss of isocntigens.

Mild Dysplasia :

Twelve cases of mild cervical dysplasia were studied. None showed complete loss of isocntigen. In 3 cases (25.0%) antigenic status was just normal, in 5 cases (41.6%), partial loss of isocntigen had occurred and rest of the 4 cases showed equivocal reaction.

However, partial loss of isocantigens has been shown in all the 8 cases of mild cervical dysplasia studied by Songfiglio and Reinberg (1976). In Lill et al's series (1978) of 34 cases 45.8% cases revealed normal antigenic expression, 25% cases showed partial loss and 29.1% cases showed complete antigenic loss.

Moderate dysplasia :

The present work incorporated 8 cases of moderately dysplastic changes of cervix. H&AR findings ranged from strongly positive (++) in 25% cases to negative (-) in 12.5% cases. Partial loss of isocantigens was found in 25% and equivocal reaction in 37.5% cases. Similarly partial loss of isocantigens in cases of moderate cervical dysplasia has also been reported in 100% cases (Songfiglio and Reinberg, 1976) and in 39% cases (Lill et al, 1976). Complete loss of isocantigens has also been reported in 29.5% cases and no loss in 41.5% cases (Lill et al, 1976).

Severe Dysplasia :

Out of 15 cases presently studied, none showed normal antigenic status. One case showed complete loss of isocantigens and the rest of the cases showed partial loss in 2 cases and equivocal reaction in another 2 cases. Songfiglio and Reinberg (1976) in their series

of 10 cases demonstrated partial loss in 9 cases and complete loss of isocytogen in one case of severe dysplasias. In another study carried out by Lill et al (1976) out of total 27 cases of severe dysplasia studied, No loss of isocytogen was found in 25 cases, 11 cases showed complete loss and partial loss was encountered in rest of the 11 cases.

So no definite pattern of antigenic expression can be defined comparable with morphological grading of dysplasia (Fig. No. 12). May be the diminished or lost expressibility indicating functional dedifferentiation of dysplastic lesion does not run parallel to morphological dedifferentiation.

3. Invasive carcinoma of uterine cervix.

Total of 46 cases of invasive carcinoma of uterine cervix were studied. HCAR showed negative reaction in (69.9%) while one case (2.2%) showed no loss of antigen. HCAR findings were equivocal in 8 cases (17.4%) and partial antigenic loss was found in 5 cases (10.9%).

Studies have also shown complete loss of antigen in 85.7% cases (Kovarik et al, 1968), 65.7% cases (Davidsohn et al, 1969) and 100% cases (Bongfiglio and Feinberg, 1976). In another study 90% cases partial and complete loss of isocytogens has also been

reported by Davidsohn and Hl (1970). Strongly positive results indicating normal antigenic expression in invasive carcinoma have also been reported by other workers also, (Kovarik et al, 1968 and Davidsohn et al, 1969).

No definite explanation for such reaction could be found. This might be possible that this was due to coexisting infection by *Escherichia coli* 068 which is antigenically similar to blood group antigens.

In Microinvasive carcinoma :

Fortunately, present study included one case of microinvasive carcinoma and that showed equivocal NCAR.

Well differentiated carcinoma :

All the 6 cases studied showed loss of isoantigen of varying degree.

Moderately differentiated carcinoma :

NCAR indicated partial to complete loss of isoantigen in all 13 cases of moderately differentiated carcinoma.

Poorly differentiated carcinoma :

Total of 24 cases were studied and NCAR showed partial to complete loss of isoantigens including one case showing no antigenic loss.

Unfortunately, so far, relationship of isoen-
tigenic loss with carcinomas of different grades have
not been studied by any worker, consequently results
of the present study are yet to be compared.

Analysis of the results of NCAR show significant
preponderance of negative or weaker NCAR in cases of
carcinoma. The number of cases of well differentiated
carcinoma showing partial loss was more than the
number of cases of poorly differentiated carcinoma
showing same type of reaction and number of moderately
differentiated carcinoma was inbetween the two.
Equivocal reaction was also were common in well
differentiated carcinoma as compared to poorly differ-
entiated one. On the contrary complete loss of antigen
was more frequent in poorly differentiated carcinoma
than in moderately and well differentiated (Fig. No. (5).

Low density in the distribution of indicator
RBC and patch pattern (equivocal reaction) in NCAR
can be explained by the heterogeneity of cellular
population in early carcinoma with resulting variation
in ability to produce or to store isoen-
tigenic. It is difficult to explain strongly positive ('++') reaction
in carcinoma and in such cases examination of additional
biopsy sections may be rewarding by finding of a

negative reaction, as loss of isocytigen need not necessarily take place in entire carcinoma at the same time.

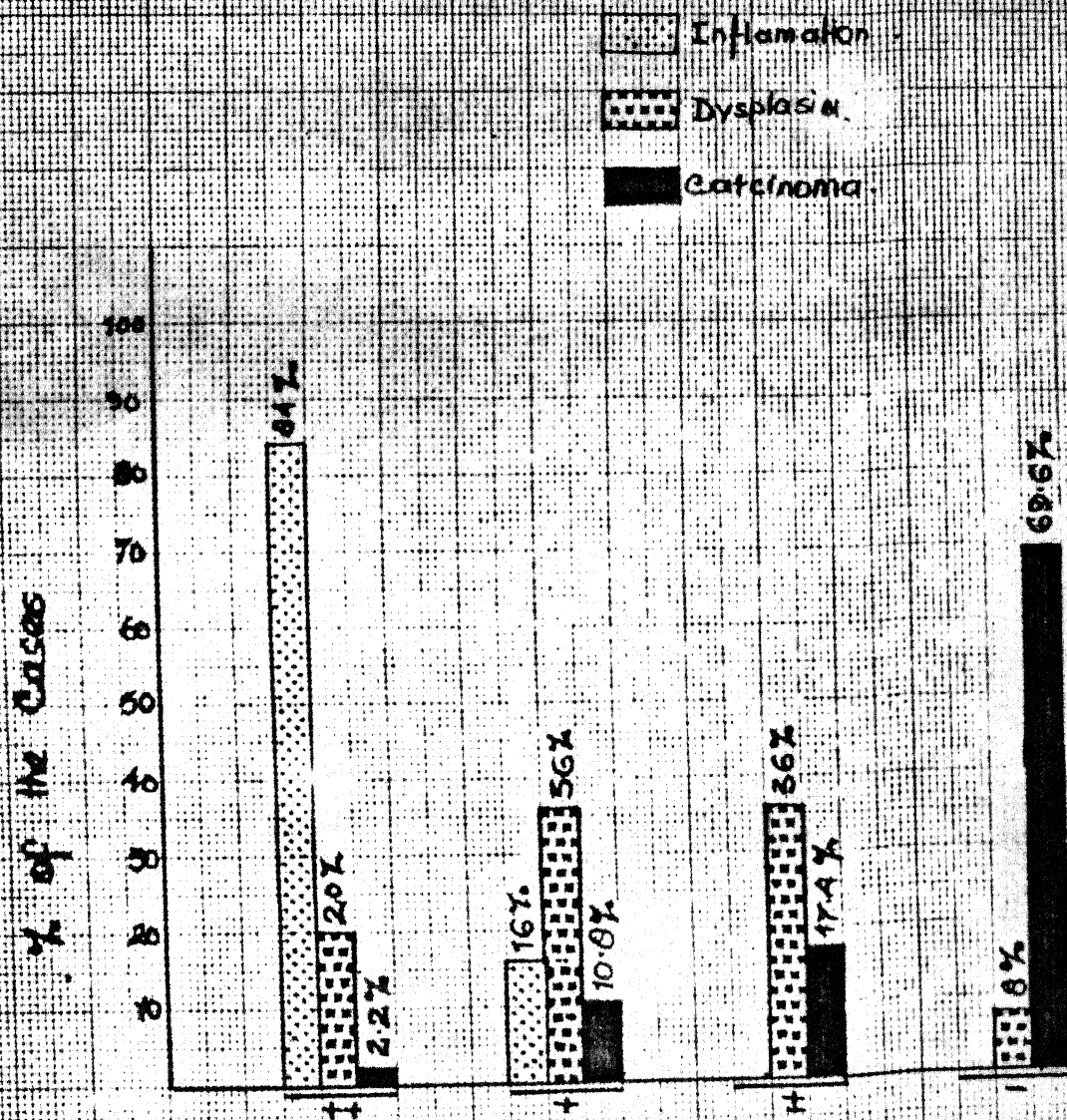
II. IMMUNOFLUORESCENCE STAINING RESULTS IN CERVICAL LESIONS :

Immunofluorescence staining was done in 55 cases of cervical lesion consisting of 45 test cases of carcinoma and dysplasia and 10 cases of chronic cervicitis serving as control. (Fig no. 14)

Comparison between the results of NCAR and IF staining revealed that both were very much alike. All the cases showing '++' and '+' reactions in NCAR gave '+' and '-' results in IF respectively, without any single exception. Most of the cases showing '+' reaction in NCAR also appeared as '+' in IF staining with the exception of 1 case of moderately differentiated carcinoma, 2 cases of poorly differentiated carcinoma and 1 case of chronic cervicitis in which IF staining was negative. IF staining was negative in cases showing '+' reaction in NCAR with the exception of one case of mild dysplasia where IF staining was positive. So this may be suggested that in cases of equivocal (+) NCAR results, IF staining can be of help to decide if isocytigens were present or not, thus confirming the nature of the cervical neoplasia.

The changes from positive to negative NCAR and IF finding may be the result of immunologic dedifferentiation and it would not be surprising to find different stages of dedifferentiation side by side. The decrease and loss of antigen may be the result of decrease in ability of epithelial cell to produce them or to store them or change in cellular membrane or change in their demonstrability by NCAR or IF or, a combination of these or any other as yet unknown factor.

M.C.A.R. IN INFLAMMATIONS, DYSPLASIAS & CARCINOMAS OF UTERINE CERVIX



Grades of M.C.A.R.

FIG. NO. 11

M.CAR IN DYSPLASIAS OF UTERINE CERVIX

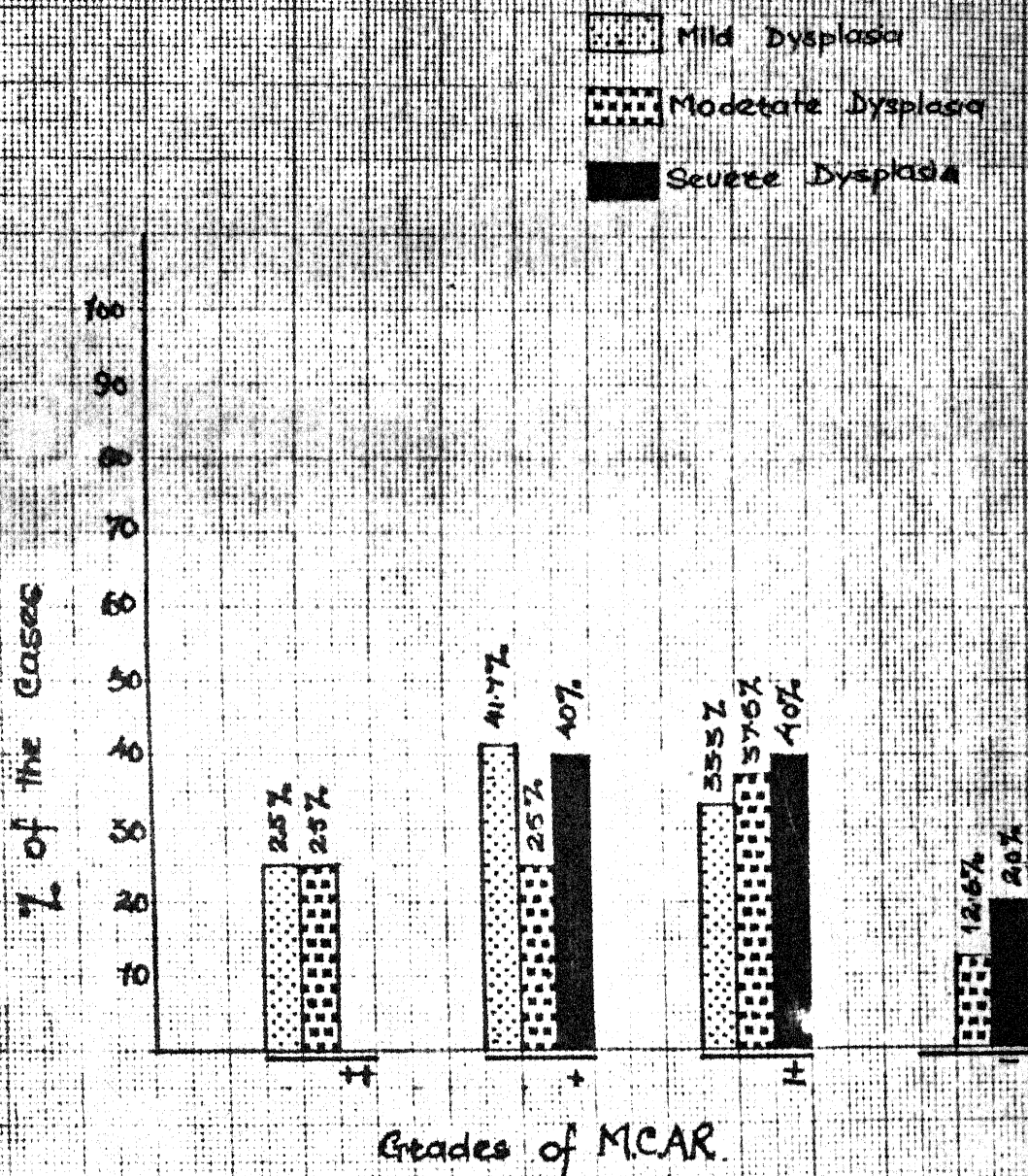






FIG. NO. 12.

MICAR IN INVASIVE CARCINOMAS OF UTERINE CERVIX

-  Micro-invasive Carcinoma.
-  Well differentiated Carcinoma.
-  Moderately differentiated Carcinoma.
-  Poorly differentiated Carcinoma.

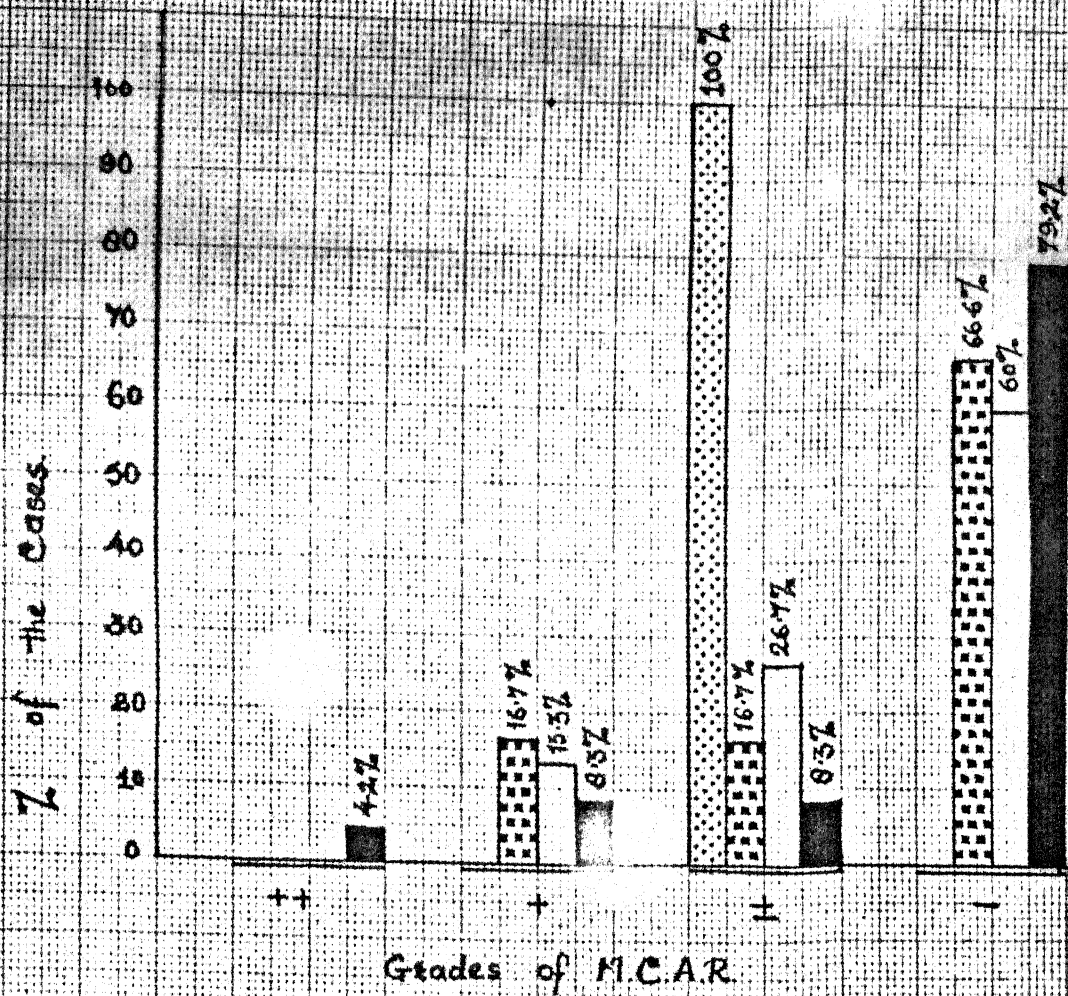



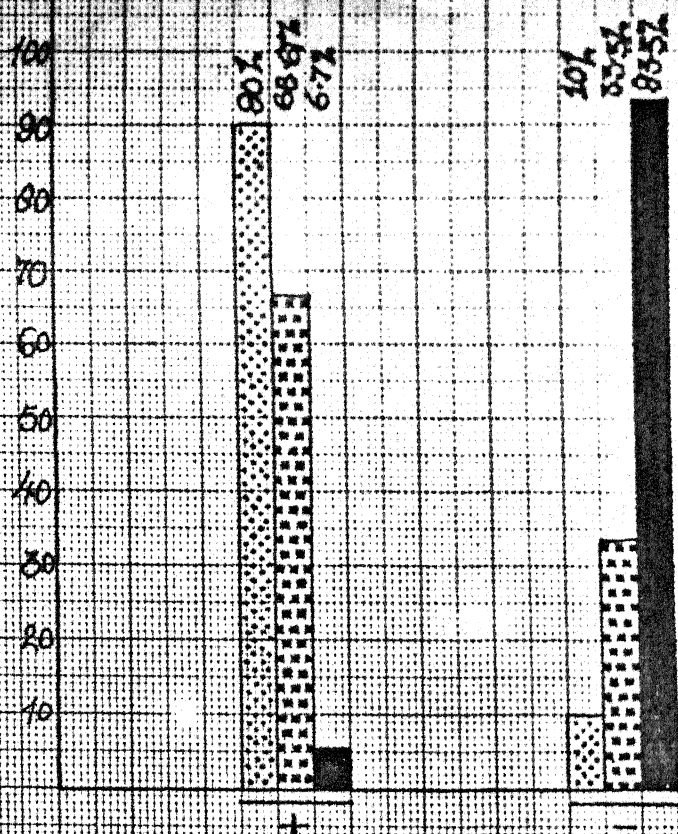


FIG. NO. B.

IF STAINING RESULTS IN INFLAMMATORY, DYSPLASTIC AND CARCINOMATOUS LESIONS OF UTERINE CERVIX.

 Inflammation
 Dysplasia
 Carcinoma



Results of P.P. Staining

FIG. No. 14

CONCLUSIONS

CONCLUSIONS

The present work consists of study of ABO(H) isoenzymes in cases of cervical neoplasia including squamous cell carcinoma and dysplasia, of cases of chronic cervicitis as well as a control group for the study.

The peak incidence of carcinoma and dysplasia was found to be in 35-49 years of age.

No statistically significant relationship between the ABO blood groups and carcinoma and dysplasia of the uterine cervix was found.

The sections prepared from old and fresh paraffin blocks of the cases selected for the study were subjected to mixed cell agglutination reaction (MCAR) or specific red cell agglutination (SRCA) reaction and immunofluorescence (IF) staining techniques.

In cases of chronic cervicitis MCAR revealed that ABO (H) isoenzymes were normally present in all the layers of lining epithelium of ectocervix, endocervix, endocervical glands and endothelial lining of blood vessels. No isoenzyme could be demonstrated in connective tissue and basal layer of ectocervix was always negative.

In cases of carcinoma, MCAR indicated complete loss of isoenzymes in 69.4% of the cases whereas 10.8% cases showed partial loss of isoenzymes and 17.4% cases showed equivocal reaction.

Results of NCAR in cases of cervical dysplasia suggested that isoeantigens may or may not be lost and antigenic loss may vary from partial to complete.

NCAR findings were supported by the results of IF staining and in cases of equivocal (+) NCAR, immunofluorescence studies were confirmatory.

NCAR was found to be more sensitive than IF technique as it could demonstrate even the partial loss of isoeantigens. On the other hand IF was more specific than NCAR since in cases where NCAR was equivocal (+), exact antigenic status could be defined by immunofluorescence.

Loss of isoeantigens from tissue in dysplasia and carcinoma may be regarded as a feature of functional dedifferentiation associated with neoplastic transformation. Though the presence of isoeantigens in tissues does not exclude malignancy but the loss of antigen may be regarded as an indicator of malignant lesions and premalignant lesions with a high potential for malignant transformation.

Follow up studies should be taken in order to determine whether there is a correlation between presence or absence of antigen - firstly in dysplastic lesions of uterine cervix and their progression to invasive carcinoma and secondly in malignant lesions of

uterine cervix and the formation of distant metastasis and prognosis.

The study of isoantigens in cervical lesions may serve as a tribute to improve detection of cervical neoplasia in its precancerous and developmental stages.



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PROFORMA

Old/Fresh

**Title of the thesis :- Study of isocitogens in
cervical neoplasia by NCAR
and immunofluorescence
technique.**

CLINICAL DATA

Patient's name :

Age and sex :

Ward/bed:

Address :

Clinical diagnosis :

Brief clinical notes :

Specimen sent

removed on

PATHOLOGICAL DATA

Patients blood group :

Known/Unknown

A, B, O, AB

Histopathology No.

date :

Histopathological diagnosis :

NCAR Findings :

Immunofluorescence staining results :
